Fig. 1

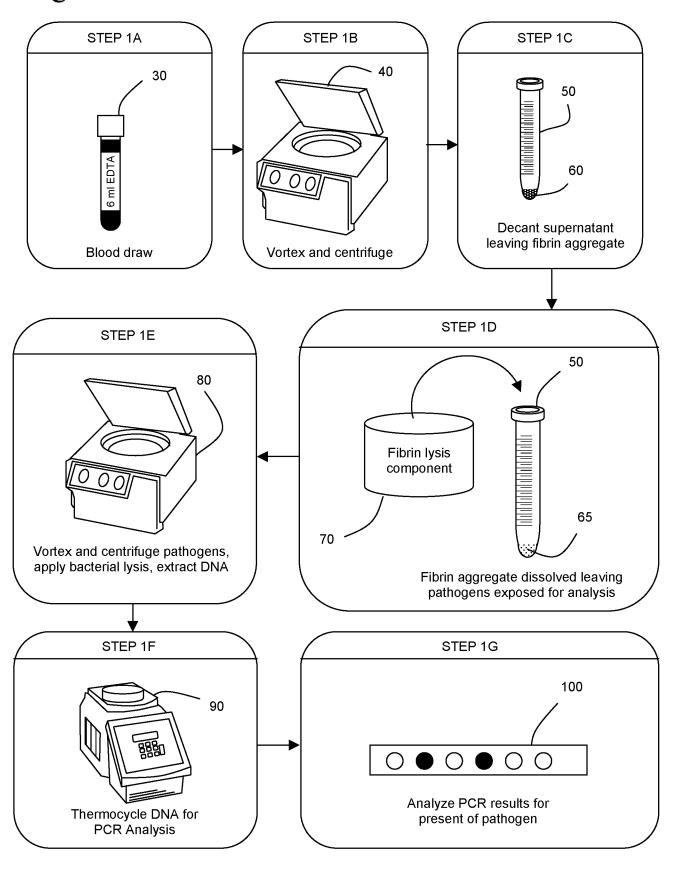


Fig. 2

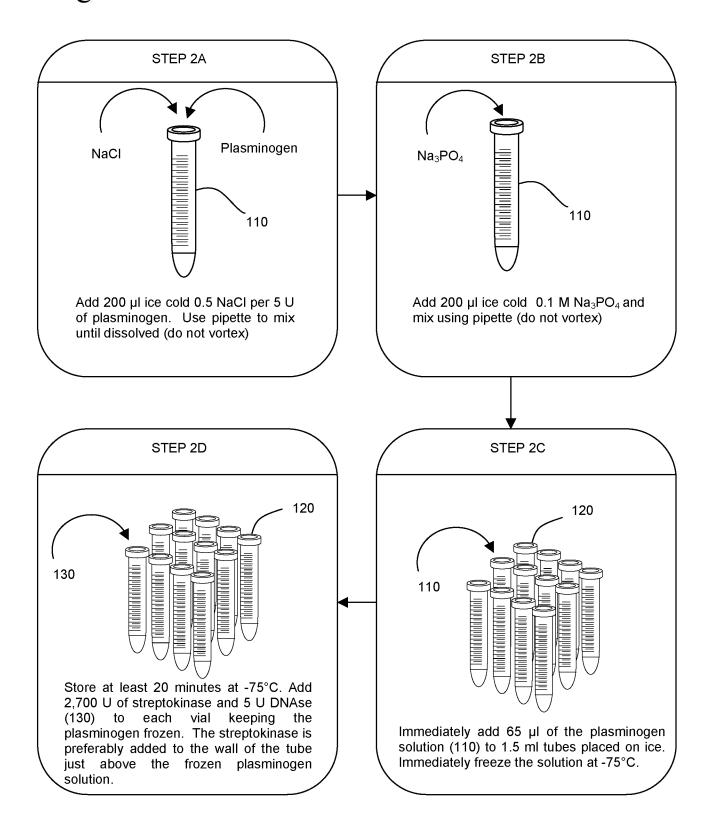


Fig. 3

Bacillus anthracis Blood Protocol Data Set

Cample Number	nVO2 Drimor /	Genomic Primer /	۸۰۰۰	Comments on
Sample Number	pXO2 Primer /		Ave.	Comments on
	Probes - Crossing	Probes - Crossing	Calculated	Sample Type All
	Point on Light Cycler	Point on Light Cycler	CFU/ 6 ml	Samples Tested 2
			of blood	Days Post Spiking
M3200253BA1	36.75	37.76	13.75	Spiked Positive
				·
M3200253BA2	36.59	37.86	13.75	Spiked Positive
M3200253BA3	35.97	38.10	13.75	Spiked Positive
M3200253BA4	37.26	39.53	13.75	Spiked Positive
M3200253BA5	35.36	40.11	13.75	Spiked Positive
M3200253BA6	36.35	45.19	13.75	Spiked Positive
M3200253BA7	36.62	38.64	13.75	Spiked Positive
M3200253BA8	37.04	39.51	13.75	Spiked Positive
M320020BA9	0.00	0.00	0.00	Blank
M/3200226BA1	37.16	39.35	1.38	Spiked Positive
M/3200226BA2	36.79	40.28	1.38	Spiked Positive
M/3200226BA3	37.92	39.94	1.38	Spiked Positive
M/3200226BA4	37.49	40.16	1.38	Spiked Positive
M/3200226BA5	39.66	40.26	1.38	Spiked Positive
M/3200226BA6	39.31	41.19	1.38	Spiked Positive
M/3200226BA7	38.48	40.73	1.38	Spiked Positive
M/320020BA8	0.00	0.00	0.00	Blank

Fig. 4

Bacillus anthracis Blood Protocol Data Set: Comparison of Blood from Two Different Individuals and Evaluation of Blood Sample Age

Sample Number	pXO2 Primer / Probes - Crossing Point on Light Cycler	Genomic Primer / Probes - Crossing Point on Light	Ave. Calculated CFU/ 6 ml of blood	Comments on Sample Type All Samples Extracted 84
		Cycler	oi biood	Days Post Spiking
V210253BA1	37.73	39.81	10.5	Blood Donor #1
V210253BA2	36.74	39.05	10.5	Blood Donor #1
V210253BA3	36.51	37.99	10.5	Blood Donor #1
V210253BA4	38.12	39.79	10.5	Blood Donor #1
V21020BA5	0.00	0.00	0.00	Blank
M210253BA1	37.86	39.81	2.25	Blood Donor #2
M210253BA2	37.84	39.22	2.25	Blood Donor #2
M210253BA3	37.24	38.52	2.25	Blood Donor #2
M210253BA4	38.68	39.33	2.25	Blood Donor #2
M21020BA5	0.00	0.00	0.00	Blank

Fig. 5

Bacillus anthracis Blood Protocol Data Set: Evaluation of Blood Protocol by a Department of Health Laboratorian

Sample Number	pXO2 Primer / Probes -	Genomic	Ave.	Comments on
	Crossing	Primer /	Calculated	Sample Type:
	Point on Light Cycler	Probes -	CFU/ 6 ml	All Blood
		Crossing	of blood	Samples Same
		Point on Light		Batch as in Table
		Cycler		1
M3200256BA1L	38.81	39.93	13.75	Spiked Positive
M3200256BA2L	36.10	39.26	13.75	Spiked Positive
M/3200223BA3L	36.77	38.58	1.38	Spiked Positive
M320020BA4L	0.00	0.00	0.00	Blank

Fig. 6

Yersinia pestis Blood Protocol Data Set

Sample Number	YP 2	YP 9	YP12	YP 16	Ave.	Comments on
	Primer /	Primer /	Primer /	Primer /	Calculat	Sample Type All
	Probes -	Probes -	Probes -	Probes -	ed CFU/	Samples
	Crossing	Crossing	Crossing	Crossing	6 ml of	Extracted 2
	Point on	Point on	Point on	Point on	blood	Days Post
	Light	Light	Light	Light Cycler		Spiking
	Cycler	Cycler	Cycler			
M3180251EYP1	0.00	0.00	0.00	37.97	12.0	Spiked Positive
M3180251EYP2	0.00	47.01	0.00	0.00	12.0	Spiked Positive
M3180251EYP3	41.56	0.00	0.00	40.29	12.0	Spiked Positive
M3180225EYP4	0.00	0.00	0.00	38.98	24.0	Spiked Positive
M3180225EYP6	40.20	44.01	39.66	37.60	24.0	Spiked Positive
M3180251FYP7	0.00	46.15	0.00	39.79	48.0	Spiked Positive
M3180251FYP8	40.48	43.59	41.70	35.47	48.0	Spiked Positive
M3180251FYP9	40.20	41.88	38.67	34.23	48.0	Spiked Positive
M318020YP10	0.00	0.00	0.00	0.00	0.00	Blank

Fig. 7

Setup of Extraction Reagents

STEP 7A

Locate a work surface not normally exposed to bacterial DNA aerosols. Wipe down the work surface with a freshly made 10% bleach solution if gross bacterial DNA contamination could potentially exist. On this work surface open a ROCHE MAGNA PURE LC DNA ISOLATION KIT III.

STEP 7B

Disposable serologic pipettes are adequate for dispensing Roche Kit III reagent volumes of > 0.5 ml. If a micropipette is used be sure to wipe the barrel with 10% bleach if that pipette has possibly been used for a previous DNA extraction.

STEP 7C

Refer to the kit insert if precipitates are observed in the ROCHE KIT III reagent bottles. The kit is stored at room temperature (15-25°C). The reagent bottles are numbered and color-coded.

STEP 7E

While using the relatively DNA free work surface aliquot into 15 ml polypropylene tubes N + 1 the amount of Wash Buffers I, II, III, Lysis Binding Buffer, and acetone needed for the samples being tested. Aliquot 300 μ l per sample of the Bacterial Lysis Buffer. The remaining 68 μ l (from the total indicated above) will be used in the next step (7F). Vortex thoroughly and aliquot N + 2 of the required amount of Magnetic Glass Particles and elution buffer into 1.5 ml tubes.

STEP 7D

The Lysis Binding Buffer contains guanidine isothiocyanate. The Wash Buffer I contains guanidine hydrochloride. These compounds are irritants. Always wear gloves and follow laboratory Chemical Hygiene protocols for spills, exposure, and disposal of these reagents. Read the ROCHE product insert.

STEP 7F

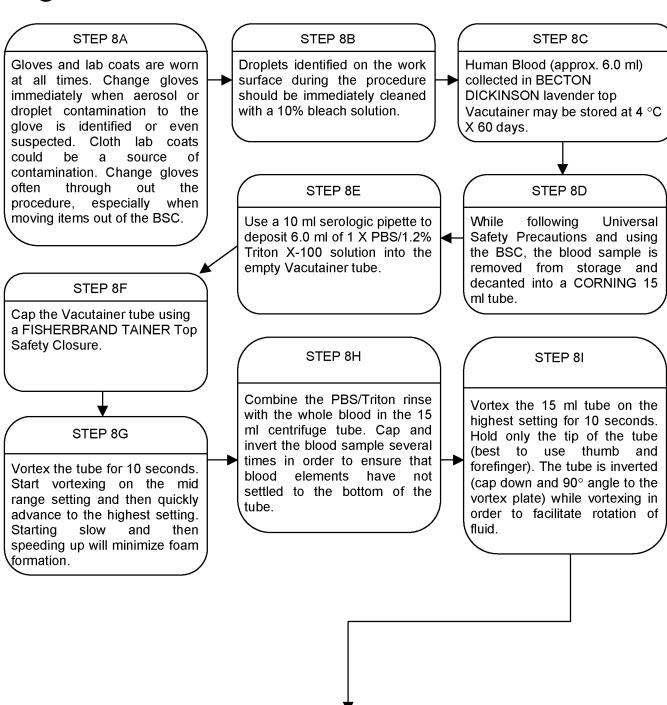
Prepare the Proteinase K solution by adding 600 μ l of Elution the Buffer to dried Proteinase K and mix by swirling with at least 20 rotations. Store the solution at room temperature while stock is in use. While not in use, the solution may be stored at 2-8°C for up to 4 weeks or at –15 to -20°C for up to 12 months. If frozen, the solution should be thawed only once.

STEP 7G

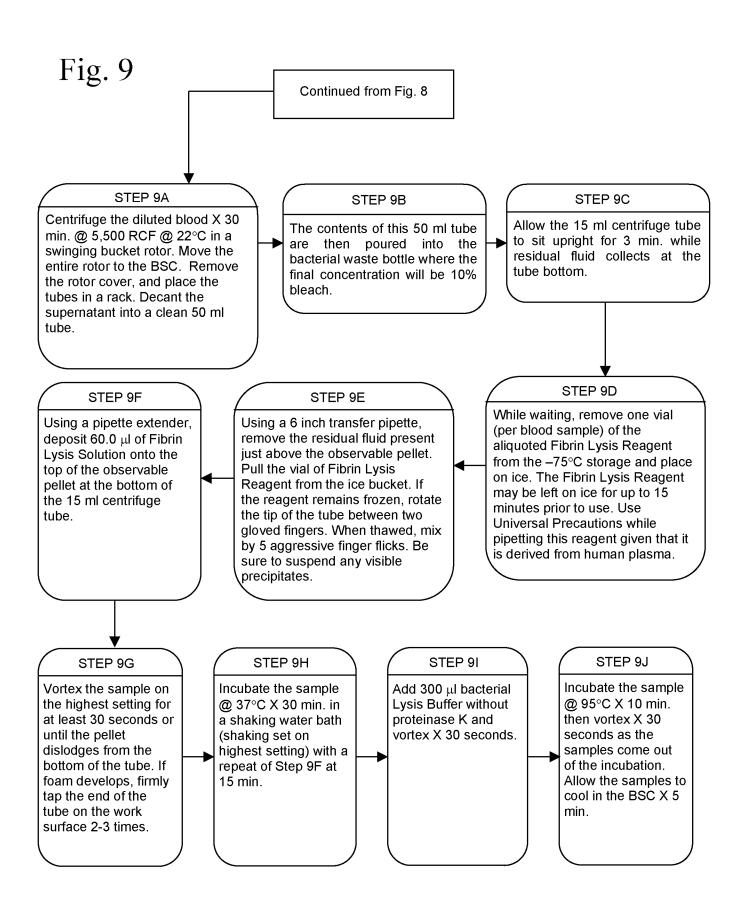
The Proteinase K Lysis Buffer is composed of Proteinase K and Bacterial Lysis Buffer. A 68.0 μ l volume of Bacterial Lysis Buffer and 32.0 μ l of Proteinase K solution are combined per sample. Multiply sample number + 1 by the each of the above values. Keep this calculation ready for use during nucleic acid extraction.

Bacterial Recovery and Fibrin Lysis

Fig. 8



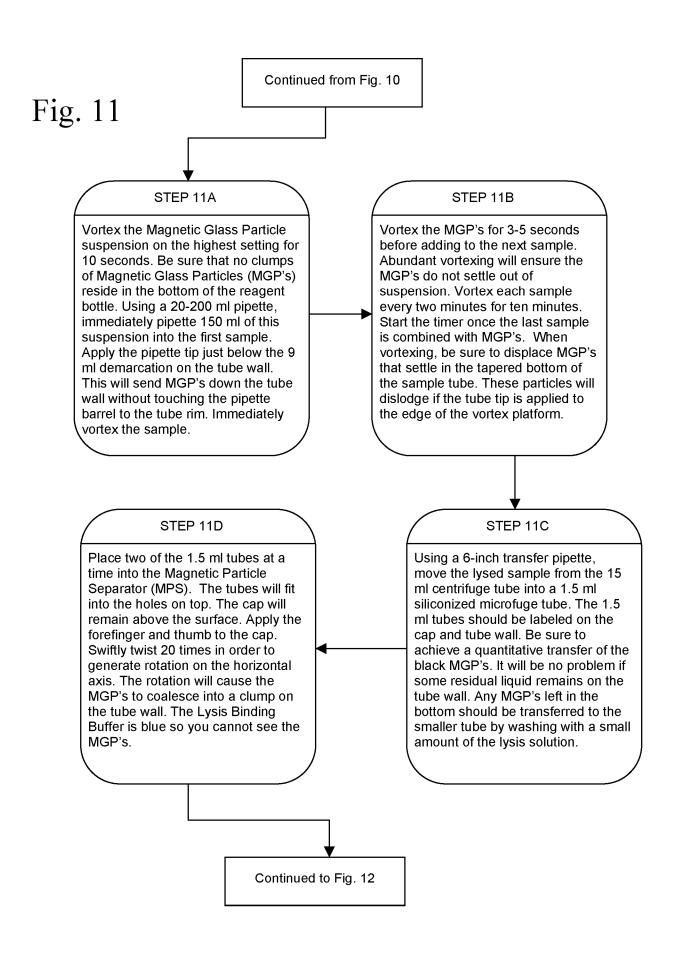
Continued to Fig. 9



Bacterial Lysis and Nucleic Acid Extraction

STEP 10A STEP 10B While waiting during the 5 minute cool down in Working inside the BSC and Step 9J of Fig. 9, combine the calculated using a 100-1000 ml volumes of Proteinase K and Bacterial Lysis micropipette, add 100.0 ml of Buffer (indicated in Step 7G of Fig. 7). Swirl or Proteinase K Bacterial Lysis invert (do not vortex) the mixture 20 times. The Buffer (made in Step 10A) to size of tube used will depend on the volume the sample material residing at made. One ml or less should be made in a 1.5 the bottom of each 15 ml ml tube. Larger volumes should be made in a 50 polypropylene centrifuge tube. ml tube in order to facilitate swirling without The exposed micropipette barrel (part not covered by the reagent loss on tube walls. Store this Proteinase K Lysis Buffer at room temperature disposable pipette tip) should remain at least 1 cm above the while the stock is in use. Dispose of any unused portion according to Laboratory Chemical lip of the 15 ml tube. Hygiene practices STEP 10C STEP 10D Vortex the sample tube for 30 The samples are moved to a seconds on the highest nucleic acid extraction area setting. Incubate the sample in preferably where there exists 100% a 65°C water bath for 30 exhaust and or UV light treatment. minutes with vortexing every Clinical sample DNA should not be 10 min. X 30 min. Incubate the extracted directly next to areas sample in a 95°C water bath where amplified DNA, bulk for 10 minutes. As samples extraction reagents or bulk come out of the 95°C water amplification reagents are stored bath vortex the contents for 30 and or manipulated. Add 500 ml of seconds on the highest setting Lysis Binding Buffer to each allow the samples to cool for 5 sample and vortex for 10 seconds minutes at room temperature. on the highest setting.

Continued to Fig. 11



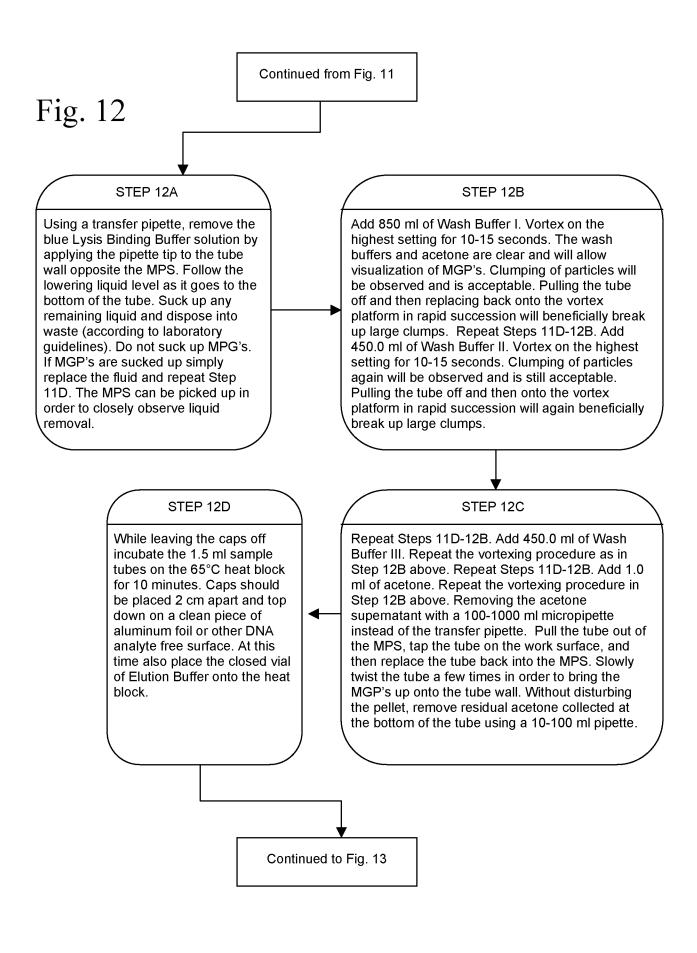


Fig. 13

Continued from Fig. 12 STEP 13A STEP 13B Apply 50 ml of warmed Elution Incubate the capped samples at 65°C for 10 minutes in the Buffer onto the MGP's at the bottom of the 1.5 ml tubes. Cap the tube heat block. Vortex the DNA and vortex on the highest setting for samples for 10 seconds on the mid range setting then 10 seconds. Start vortexing on the high range setting and then at the spin at 16,000 RCF X 3 end of 10 seconds, slowly reduce minutes. The MGP's will form speed to the lowest setting. Ending a firm pellet. Using a 50 ml the vortex with a speed reduction setting, pipette the will minimize droplet deposition in supernatant into a clean 1.5 the upper aspect of the microfuge ml tube and proceed to PCR tube. Some samples will appear to or store the samples at -20°C. not vortex. This condition is acceptable. STEP 13C For the CDC Bacillus anthracis based oligos. PCR testing should be done using sample DNA dilutions of 1:15 and at least a 20ml reaction final volume. For the CDC Yersinia pestis based oligos, a 1:20 dilution of sample DNA should be used in at least a 35 ml reaction

final volume.

Fig. 14a

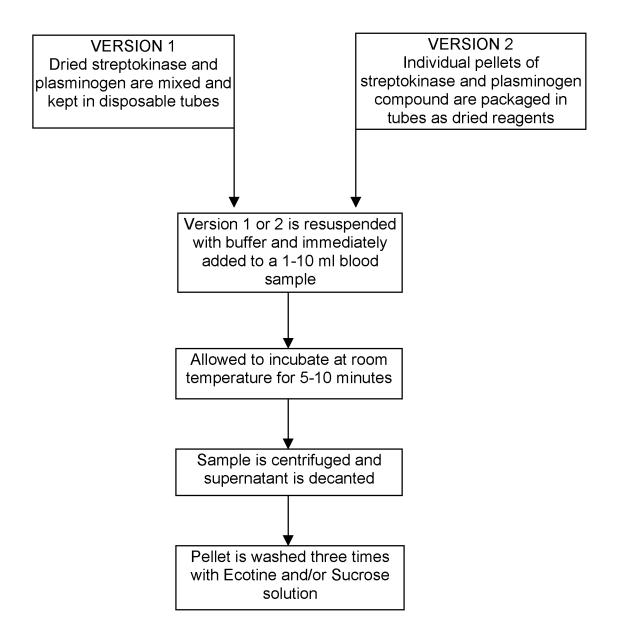


Fig. 14b

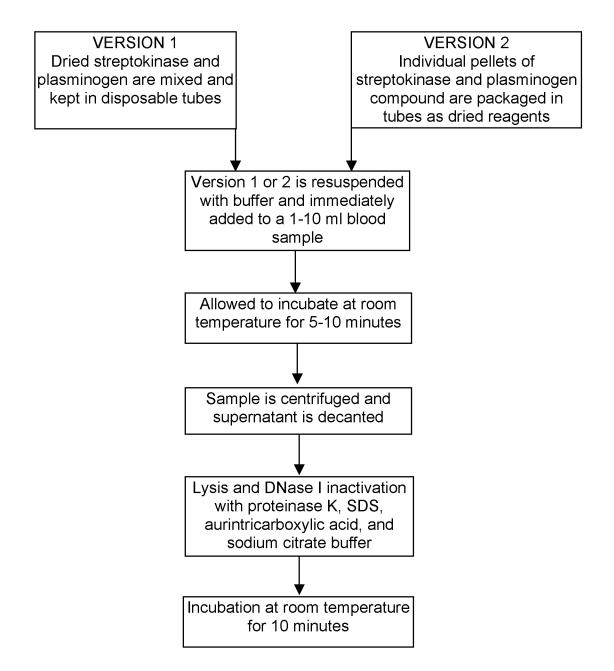


Fig. 15

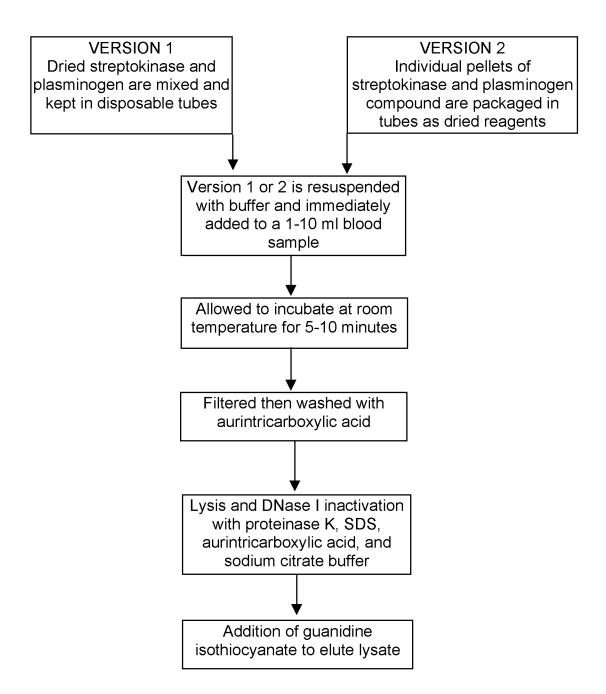


Fig. 16a

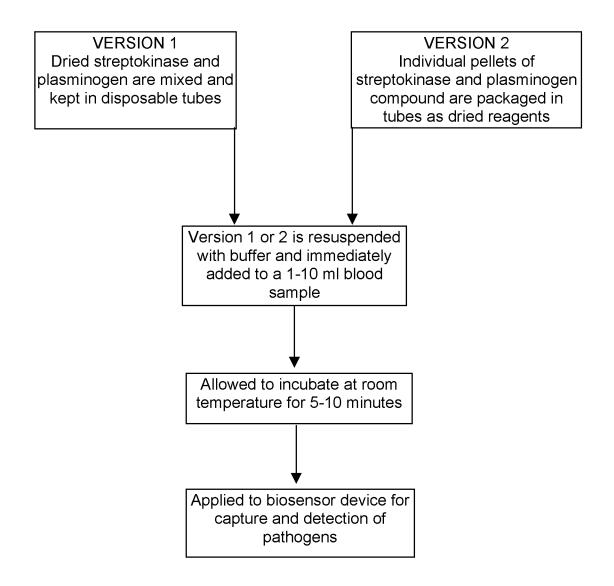


Fig. 16b

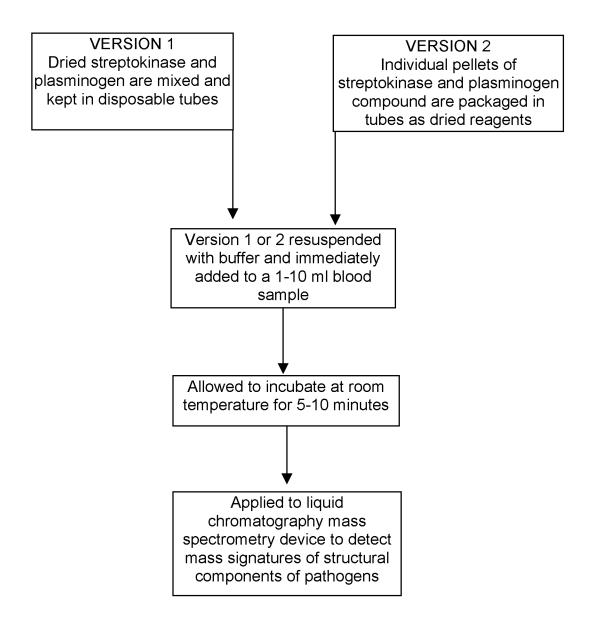


Fig. 17

Noise band crossing points for blood samples spiked with B. anthracis and processed with plasminogen, streptokinase, phospholipase A_2 , DNase I, and lipase with centrifugation or filtration

-	Centrifugation				<u>Filtration</u>				
Amount B. anthracis Seeded (cfu)	Noise Band Cro Points	ossing	Mean	Std. Dev.	Noise Band Crossing Points		Mean	Std. Dev.	
$ \leq 0.01 $ $ \leq 0.01 $ $ \leq 1.0 $ $ \leq 1.0 $ $ \leq 2.0 $ $ \leq 2.0 $ $ \leq 5.0 $ $ \leq 5.0 $ $ \leq 50.0 $	41.93 38.11 37.53 36.24 36.45 38.15	40.31 40.47 40.36 37.90 38.49	41.12 40.47 39.24 37.22 37.70	0.87 1.09	40.33 40.36 37.90 36.45 35.75 35.24	39.89 37.79 37.70 36.09 34.12 34.18	37.69 37.79 36.81 34.98 34.68	40.11 37.79 39.03 37.80 36.45 34.95 34.70	0.10 0.36 0.82 0.53

Fig. 18

Sedimentation and solublization of tissue aggregates from 6 ml blood samples exposed to various detergent and enzyme treatments

Enzyme treatments in a PBS/Triton X-100 buffer

	Triton X-100 in PBS	Pl. ^c 1U	Ph. ^b	P1.° 1U Ph. ^b	Dn. ^a 1mg	Dn. ^a 1 mg Ph. ^b	Dn. ^a 1 mg Pl. ^c 1U Ph. ^b
% Observable pelleted tissue aggregate post centrifugation	100	100	100	100	90	10	10
Time (min) to solubilization of visible tissue aggregate in BLB ^d	> 360	> 60	> 60	> 60	< 10	< 0.5	< 0.5

^a DNase I from the Roche MagNa Pure LC DNA Kit III

b Phospholipase A₂
^c Plasminogen and 10K U streptokinase
^d Bacterial Lysis Buffer from the Roche MagNa Pure LC DNA Kit III

Fig. 19

Filtration characteristics of 6 ml blood samples exposed to various detergent and enzyme treatments

Enzyme treatments in a PBS/Triton X-100 buffer

	Triton X-100 in PBS	Dn. ^a 1mg	Dn. ^a 1 mg Ph. ^b	P1.° 5U	Pl. ^c 5U Dn. ^a 1mg Ph. ^b	Pl. ^c 5U Dn. ^a 0.2mg Ph. ^b	P1. ^c 10U Dn. ^a 0.2mg Ph. ^b
Not filterable	+	+	+				
Filterable with observable tissue aggregates				+		+	
Filterable with out observable aggregates					+		+

 $^{^{\}overline{a}}$ DNase I from the Roche MagNa Pure LC DNA Kit III b Phospholipase A_2 c Plasminogen converted to plasmin with $10 \mathrm{K}~\mathrm{U}~\mathrm{streptokinase}$